Solid-Phase Synthesis of Oxetane Modified Peptides

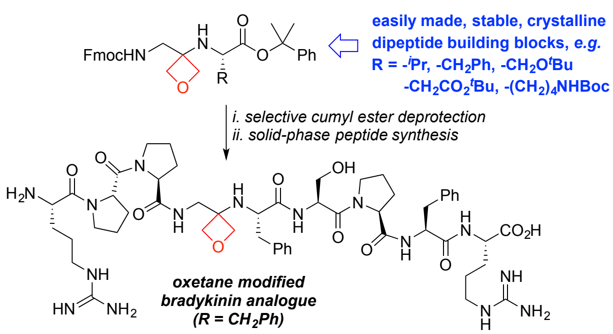
Jonathan D. Beadle,[a] Astrid Knuhtsen,[b] Alex Hoose,[b] Piotr Raubo,[c] Andrew G. Jamieson*[b] and Michael Shipman*[a]

[a] Department of Chemistry, University of Warwick, Gibbet Hill Road, Coventry, CV4 7AL, UK

[b] School of Chemistry, University of Glasgow, Joseph Black Building, University Avenue, Glasgow, G12 8QQ, UK

[c] AstraZeneca, IMED Oncology, 310 Cambridge Science Park, Milton Road, Cambridge, CB4 0WG, UK

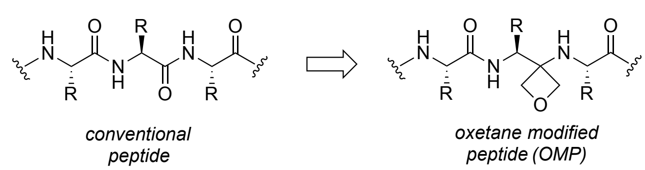
Supporting Information Placeholder



ABSTRACT: Solid-phase peptide synthesis (SPPS) is used to create peptidomimetics in which one of the backbone amide C=O bonds is replaced by a four-membered oxetane ring. The oxetane containing dipeptide building blocks are made in three steps in solution, then integrated into peptide chains by conventional Fmoc SPPS. This methodology is used to make a range of peptides in high purity including backbone modified derivatives of the nonapeptide bradykinin and Met- and Leu-enkephalin.

Despite resurgent interest in the use of peptides as drugs,[1] their development is often hampered by their poor oral bioavailability and short plasma half-lives. As a consequence, there is intense interest in the discovery of molecules that can mimic the structure and biological function of native peptides yet possess better drug-like properties.[2-4] Independently, Shipman[5] and Carreira[6] introduced a new type of peptide bond isostere in which the heterocyclic 3-aminooxetane unit was used as a replacement for one of the amide bonds (Figure 1).[7] Several features of these oxetane modified peptides (OMPs) make them of particular interest as peptidomimetics. Firstly, deletion of one of the amide bonds can improve half-lives through increased stability to proteases.[6b] Secondly, oxetanes are known to make excellent bioisosteric replacements for C=O bonds, and are commonly used in conventional, small molecule drug discovery.[8] Thirdly, the 3-aminooxetane unit can act as both a H-bond donor and acceptor, supporting the types of non-covalent interactions available to peptides. Finally, conformational changes arising from removal of the double bond character of the peptide bond change the conformational bias of OMPs,[5a] opening up new areas of peptide structural space to explore.

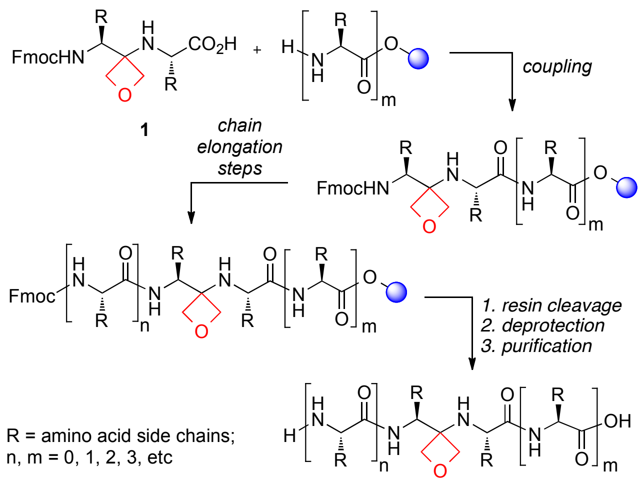
Figure 1. Oxetane Based Peptidomimetics



To date, access to OMPs has been by way of solution-based methods.[5,6] However, to study the impact of oxetane modification on the structure and properties of larger, biologically important peptides, we turned to solid-phase peptide synthesis (SPPS).[9] Our plan was to synthesize Fmoc-protected dipeptide building blocks such as **1**, then integrate them into a growing peptide chain using automated SPPS techniques (Scheme 1). Here, we describe the successful development of this methodology, and use it to make a range of OMPs including novel enkephalin and bradykinin analogues.

Initially, we sought a practical route to Fmoc-protected dipeptide building blocks in which the oxetane residue is based on glycine. Conjugate addition of the appropriate amino ester, made from **2a-i** by initial Fmoc cleavage, to 3-(nitromethylene)oxetane readily gave the addition products **3a-i** (Table 1).[5] Raney Ni reduction of **3a-i** in the presence of FmocOSu provided **4a-i** in good yields.[6] A range of protected side chain types were introduced namely: acidic, basic, polar and non-polar.

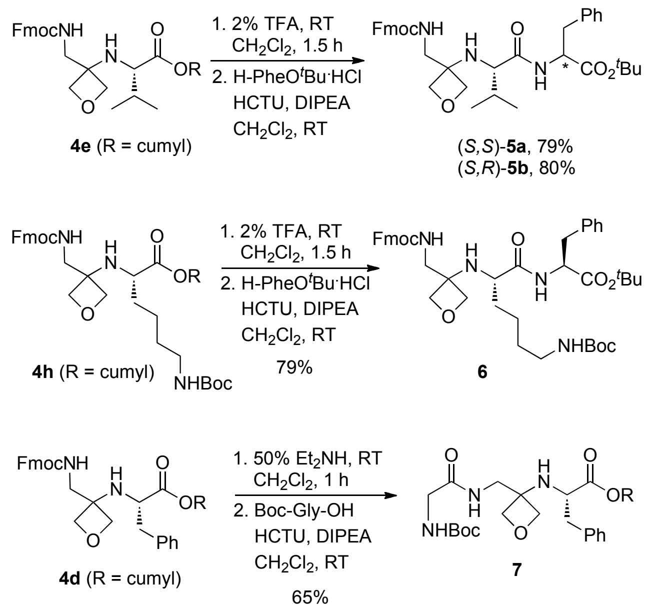
Scheme 1. SPPS Route to Oxetane Modified Peptides



The last step prior to SPPS was selective removal of the ester group (R^1); a transformation that required considerable optimization to safeguard the Fmoc and side chain protecting groups. Initially, phenylalanine derivatives containing methyl (**4a**), benzyl (**4b**), 2,4-dimethoxybenzyl (**4c**) and 2-phenylisopropyl (cumyl, **4d**) esters were studied (Table 1, entries 1-4). Cleavage of the methyl ester from **4a** using LiOH-THF/H_2O led to unwanted *N*-Fmoc cleavage. Other conditions reported to leave Fmoc groups unscathed such as Me_3SnOH[10] and NaOH/$CaCl_2$[11] also proved unsuccessful. Hydrogenolysis of benzyl ester **4b** (H_2, 10% Pd/C, MeOH) did provide the corresponding carboxylic acid in an encouraging 50% yield. However, optimization of this transformation and its extension to derivatives containing other side chains proved impractical.[12] Poor results were also observed in the attempted removal of the 2,4-dimethoxybenzyl group from **4c** using mild acid (1% TFA-CH_2Cl_2, anisole).[13] Much better outcomes were achieved with cumyl ester **4d** which was quantitively deprotected upon treatment with 2% TFA in CH_2Cl_2 for 1.5 h.[14a] On this basis, we focused our subsequent efforts on cumyl esters **4d-i**. From a practical standpoint, these derivatives proved attractive as they were bench stable, crystalline solids. Indeed, it proved most convenient to store the building blocks as the cumyl esters, then reveal the carboxylic acid using 2% TFA in CH_2Cl_2 immediately prior to SPPS. These materials were produced on up to a 3 mmol scale (see Supporting Information). Moreover, the starting cumyl esters **2d-i** were easily made from the corresponding Fmoc-protected amino acids by reaction with 2-phenylisopropyl-trichloroacetimidate (see Supporting Information).[14]

Initially, the use of the building blocks in solution-phase couplings was established (Scheme 2). Cleavage of the cumyl ester from **4e** followed by coupling with L-phenylalanine *tert*-butyl ester with O-(1H-6-chlorobenzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HCTU) and diisopropylethylamine (DIPEA) gave (*S,S*)-**5a** in excellent yield. Using D-phenylalanine *tert*-butyl ester in the same sequence provided (*S,R*)-**5b**. Analysis by ^{1}H NMR confirmed that no detectable epimerization arose during these couplings (see Supporting Information). Subjecting **4h** bearing a Boc-protected lysine to the same cleavage/coupling conditions cleanly gave **6** without evidence of side chain deprotection. Additionally, **4d** was converted to **7** by chain extension at the N-terminus. In this transformation, no products derived from acylation of the secondary amine of the 3-aminooxetane unit were isolated despite the use of 4 equiv. of the coupling partners. This observation encouraged us to explore SPPS without recourse to protection of the oxetane nitrogen atom.

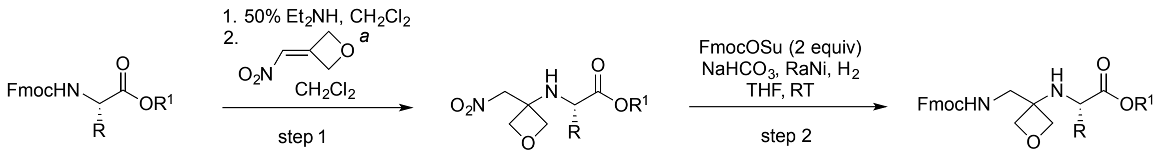
Scheme 2. Selective Cumyl Ester Deprotection and Solution Phase Couplings



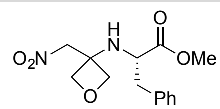
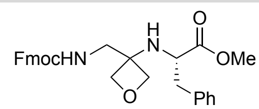
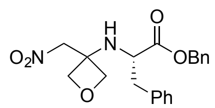
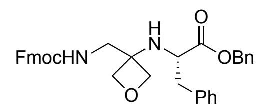
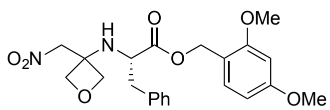
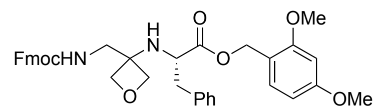
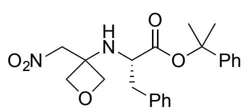
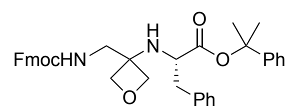
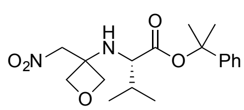
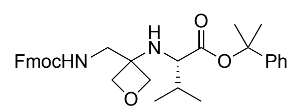
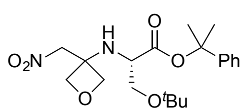
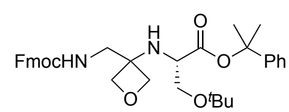
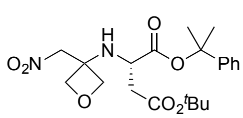
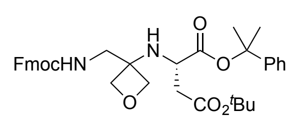
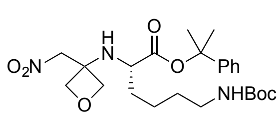
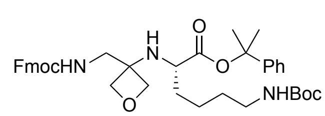
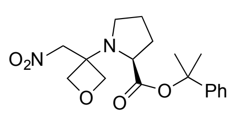
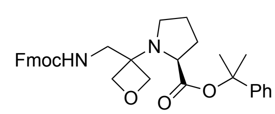
Next, we examined the use of **4d-i** in SPPS. These experiments were conducted on a 0.1 mmol scale in a Biotage Alstra microwave synthesizer using preloaded chlorotrityl resin and HCTU activation (for full details, see Supporting Information). Initially, six tetrapeptides **8-13** were synthesized using each of the building blocks, **4d-i** (Table 2, entries 1-6). Two equivalents of the building blocks were used, with a pre-treatment with 2% TFA in CH_2Cl_2 under anhydrous conditions to cleave the cumyl ester, immediately prior to coupling. Standard conditions for Fmoc removal (20% piperidine, 0.1 M Oxyma pure, DMF),[15] coupling [Fmoc-Trp-OH, HCTU, DIPEA, DMF] and resin cleavage/deprotection [TFA/triisopropylsilane(TIS)/CH_2Cl_2 (70:20:10, v/v/v)] were adopted. After reverse-phase HPLC, these tetrapeptides were isolated in high purity and acceptable yields. Pleasingly, the oxetane did not undergo ring opening under the harsh acidic conditions required for release from the resin and concomitant side chain deprotection. Encouraged by these results, we prepared oxetane modified analogues of biologically relevant peptides. First, we made **14** and **15**, analogues of opioid-binding Met- and Leu-enkephalin respectively, in which the central glycine was replaced (Table 2, entries 7 and 8).[16] Similarly, **16** was produced in good yield and excellent purity as an analogue of the vasodilator bradykinin. No erosion in peptide yield or purity was seen in the preparation of this nonapeptide, indicating that the 3-aminooxetane residue is well tolerated in repetitive rounds of coupling/Fmoc deprotection.

In summary, we have developed a practical route to oxetane modified peptides using solid-phase peptide synthesis techniques. Key findings include: (i) that cumyl-protected dipeptide building blocks **4d-i** are easily made in three simple steps and the ester selectively cleaved with 2% TFA in CH_2Cl_2; (ii) that efficient amide couplings using these building blocks can be achieved in solution or on solid-phase without protecting the secondary amine of the 3-aminooxetane residue; (iii) that oxetane containing peptides (up to 9 amino acids) can be produced in high purity using conventional SPPS methods for coupling, resin cleavage, deprotection and purification suggesting the broad applicability of this modification in peptide science. Current work is focused on using this new methodology to produce a variety of OMPs so that the impact of this backbone modification on the secondary structure, physicochemical and biological properties of the peptides can be systematically explored.

Table 1. Synthesis of Oxetane Containing Dipeptide Building Blocks

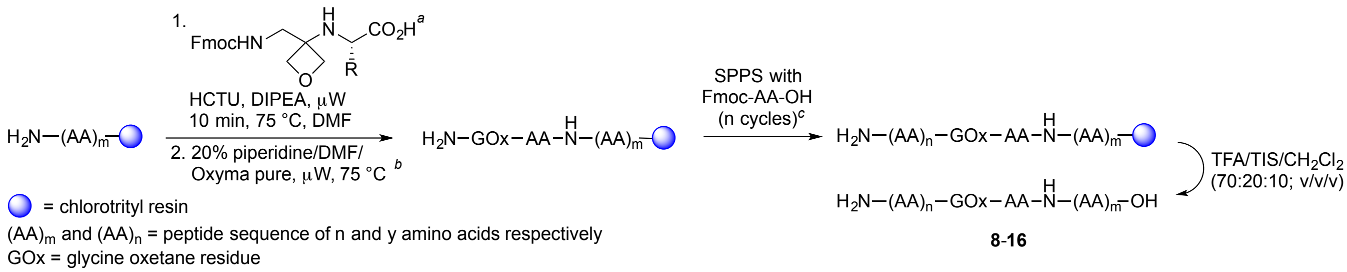


2a-i **3a-i** **4a-i**

entry	product (step 1)	structure	yield (%)[b]	product (step 2)	structure	yield (%)[b]
1	**3a**		54[c,d]	**4a**		87[f]
2	**3b**		73[c,e]	**4b**		69[f]
3	**3c**		61	**4c**		63[f]
4	**3d**		57	**4d**		58
5	**3e**		55	**4e**		76
6	**3f**		54	**4f**		78
7	**3g**		40	**4g**		77
8	**3h**		56	**4h**		71
9	**3i**		56[c]	**4i**		50

[a] Prepared *in-situ* from oxetan-3-one and nitromethane (see Supporting Information). [b] After column chromatography. [c] Made directly from α-amino ester without Fmoc deprotection. [d] Ref 5b. [e] Ref 5a. [f] Using 1.2 equiv FmocOSu.

Table 2. Solid-Phase Synthesis of Oxetane Modified Peptides



entry	building block[a]	peptide sequence[d]	purity (%)[e]	mass, mg (yield, %)	peptide content (%)[f]	HRMS calculated	HRMS observed
1	**4d**	W-*GOx-F*-A, **8**	89	10.7 (21)	75	508.2554 $[M+H]^+$	508.2531 $[M+H]^+$
2	**4e**	W-*GOx-V*-A, **9**	93	8.4 (18)	55	460.2554 $[M+H]^+$	460.2544 $[M+H]^+$
3	**4f**	W-*GOx-S*-A, **10**	81 (90)[g]	12.0 (27)	73	448.2191 $[M+H]^+$	448.2190 $[M+H]^+$
4	**4g**	W-*GOx-D*-A, **11**	81 (91)[g]	13.7 (29)	75	476.2140 $[M+H]^+$	476.2129 $[M+H]^+$
5	**4h**	W-*GOx-K*-A, **12**	93	5.0 (10)	48	489.2820 $[M+H]^+$	489.2818 $[M+H]^+$
6	**4i**	W-*GOx-P*-A, **13**	94	14.6 (32)	62	480.2217 $[M+Na]^+$	480.2213 $[M+Na]^+$
7	**4d**	Y-G-*GOx-F*-M, **14**	91	17.0 (28)	81	602.2643 $[M+H]^+$	602.2659 $[M+H]^+$
8	**4d**	Y-G-*GOx-F*-L, **15**	91	16.4 (28)	79	584.3079 $[M+H]^+$	584.3056 $[M+H]^+$
9	**4d**	R-P-P-*GOx-F*-S-P-F-R, **16**	94	25.3 (46)	63	544.8036 $[M+2H]^{2+}$	544.8014 $[M+2H]^{2+}$

[a] Treated with 2% TFA in CH_2Cl_2 for 2 h to reveal the free carboxylic acid from **4d-i** prior to coupling with the resin-bound peptide. [b] 30 s, then 3 min with fresh reagents. [c] All couplings performed at 75 °C for 10 min except arginine (60 min at RT, then 5 min at 75 °C, repeated with fresh reagents). [d] Italized residues derived from the oxetane containing dipeptide building block. [e] By reverse-phase HPLC (see Supporting Information). [f] Determined by UV spectroscopy (at 280 nm) except **16** (at 214 nm). [g] Improved to >90% by second purification.

ASSOCIATED CONTENT

Supporting Information

Experimental procedures and characterization data for all new compounds, copies of ^1H and ^{13}C NMR spectra for building blocks, and analytical HPLC traces for peptides. This Supporting Information is available free of charge on the ACS Publications website.

AUTHOR INFORMATION

Corresponding Author

* andrew.jamieson.2@glasgow.ac.uk
* m.shipman@warwick.ac.uk

ACKNOWLEDGMENT

We thank AstraZeneca for generous financial support.

REFERENCES

1. (a) Fosgerau, K.; Hoffmann, T. *Drug Discov. Today* **2015**, *20*, 122. (b) Nevola, L.; Giralt, E. *Chem. Commun.* **2015**, *51*, 3302. (c) Kaspar, A. A.; Reichert, J. M. *Drug Discov. Today* **2013**, *18*, 807.
2. Trabocchi A.; Guarna, A. *Peptidomimetics in Organic and Medicinal Chemistry: The Art of Transforming Peptides in Drugs*, John Wiley & Sons Ltd: Chichester, 2014.
3. For reviews, see (a) Qvit, N.; Rubin, S. J. S.; Urban, T. J.; Mochly-Rosen, D.; Gross, E. R. *Drug Discov. Today* **2017**, *22*, 454. (b) Avan, I.; Hall, C. D.; Katritzky, A. R. *Chem. Soc. Rev.* **2014**, *43*, 3575. (c) Liskamp, R. M. J.; Rijkers, D. T. S.; Kruijtzer, J. A. W.; Kemmink, J. *ChemBioChem* **2011**, *12*, 1626. (d) Grauer, A.; König, B. *Eur. J. Org. Chem.* **2009**, 5099. (e) Vagner, J.; Qu, H.; Hruby, V. J. *Curr. Opin. Chem. Biol.* **2008**, *12*, 292.
4. For illustrative examples, see (a) Nadon, J.-F.; Rochon, K.; Grastilleur, S.; Langlois, G.; Dao, T. T. H.; Blais, V.; Guérin, B.; Gendron, L.; Dory, Y. L. *ACS Chem. Neurosci.* **2017**, *8*, 40. (b) Busschaert, N.; Thompson, S.; Hamilton, A. D. *Chem. Commun.* **2017**, *53*, 313. (c) Krishnan, B. P.; Rai, R.; Asokan, A.; Sureshan, K. M. *J. Am. Chem. Soc.* **2016**, *138*, 14824. (d) Lee, K. J.; Lee, W. S.; Yun, H.; Hyun, Y.-J.; Seo, C. D.; Lee, C. W.; Lim, H.-S. *Org. Lett.* **2016**, *18*, 3678. (e) Wu, H.; Qiao, Q.; Teng, P.; Hu, Y.; Antoniadis, D.; Zuo, X.; Cai, J. *Org. Lett.* **2015**, *17*, 3524. (f) Barnard, A.; Long, K.; Martin, H. L.; Miles, J. A.; Edwards, T. A.; Tomlinson, D. C.; Macdonald, A.; Wilson, A. J. *Angew. Chem. Int. Ed.* **2015**, *54*, 2960. (g) Newberry, R. W.; VanVeller, B.; Raines, R. T. *Chem. Commun.* **2015**, *51*, 9624. (h) Doan, N.-D.; Hopewell, R.; Lubell, W. D. *Org. Lett.* **2014**, *16*, 2232. (i) Traoré, M.; Doan, N.-D.; Lubell, W. D. *Org. Lett.* **2014**, *16*, 3588.
5. (a) Powell, N. H.; Clarkson, G. J.; Notman, R.; Raubo, P.; Martin, N. G.; Shipman, M. *Chem. Commun.* **2014**, *50*, 8797. (b) Beadle, J. D.; Powell, N. H.; Raubo, P.; Clarkson, G. J.; Shipman, M. *Synlett* **2016**, *27*, 169.
6. (a) McLaughlin, M.; Yazaki, R.; Fessard, T. C.; Carreira, E. M. *Org. Lett.* **2014**, *16*, 4070. (b) Möller, G. P.; Müller, S.; Wolfstädter, B. T.; Wolfrum, S.; Schepmann, D.; Wünsch, B.; Carreira, E. M. *Org. Lett.* **2017**, DOI:10.1021/acs.orglett.7b00745.
7. Oxetane modification of proteins through side chain functionalization has also recently been described, see Boutureira, O.; Martínez-Sáez, N.; Brindle, K. M.; Neves, A. A.; Corzana, F.; Bernardes, G. J. L. *Chem. Eur. J.* **2017**, *23*, DOI: 10.1002/chem.201700745.
8. (a) Bull, J. A.; Croft, R. A.; Davis, O. A.; Doran, R.; Morgan, K. F. *Chem. Rev.* **2016**, *116*, 12150. (b) Burkhard, J. A.;

Wuitschik, G.; Rogers-Evans, M.; Müller, K.; Carreira, E. M. *Angew. Chem. Int. Ed.* **2010**, *49*, 9052.
9. Bodanszky M.; Bodanszky, A. *The Practice of Peptide Synthesis*, Springer-Verlag: Berlin, 1994.
10. Nicolaou, K. C.; Estrada, A. A.; Zak, M.; Lee, S. H.; Safina, B. S. *Angew. Chem. Int. Ed.* **2005**, *44*, 1378.
11. Pascal, R.; Sola, R. *Tetrahedron Lett.* **1998**, *39*, 5031.
12. When the side chain of **4b** (R = Bn) was replaced with other substituents (R = H or ^{i}Pr), cleavage of the Fmoc group was seen during hydrogenolysis. We speculate that the success witnessed with **4b** (R = Bn) arises from its insolubility in MeOH. During hydrogenolysis, the carboxylic acid rapidly precipitates preventing further Fmoc cleavage.
13. McMurray, J. S. *Tetrahedron Lett.* **1991**, *32*, 7679.
14. (a) Yue, C.; Thierry, J.; Potier, P. *Tetrahedron Lett.* **1993**, *34*, 323. (b) Respondek, T.; Cueny, E.; Kodanko, J. J. *Org. Lett.* **2012**, *14*, 150.
15. Subirós-Funosas, R.; El-Faham, A.; Albericio, F. *Biopolymers (Peptide Science)* **2012**, *98*, 89.
16. The solution-phase synthesis of Leu-enkephalin analogue **15** has previously been described, see ref. 6b.